Comparison of doxorubicin-cyclophosphamide with doxorubicin-dacarbazine for the adjuvant treatment of canine hemangiosarcoma

Finotello, R ; Stefanello, D ; Zini, E ; Marconato, L

Abstract: Canine hemangiosarcoma (HSA) is a neoplasm of vascular endothelial origin that has an aggressive biological behaviour, with less than 10% of dogs alive at 12-months postdiagnosis. Treatment of choice consists of surgery followed by adjuvant doxorubicin-based chemotherapy. We prospectively compared adjuvant doxorubicin and dacarbazine (ADTIC) to a traditional doxorubicin and cyclophosphamide (AC) treatment, aiming at determining safety and assessing whether this regimen prolongs survival and time to metastasis (TTM). Twenty-seven dogs were enrolled; following staging work-up, 18 were treated with AC and 9 with ADTIC. Median TTM and survival time were longer for dogs treated with ADTIC compared with those receiving AC (>550 versus 112 days, P = 0.021 and >550 versus 142 days, P = 0.011, respectively). Both protocols were well tolerated, without need for dose reduction or increased interval between treatments. A protocol consisting of combined doxorubicin and dacarbazine is safe in dogs with HSA and prolongs TTM and survival time.

DOI: https://doi.org/10.1111/vco.12139

Originally published at:
Finotello, R; Stefanello, D; Zini, E; Marconato, L (2017). Comparison of doxorubicin-cyclophosphamide with doxorubicin-dacarbazine for the adjuvant treatment of canine hemangiosarcoma. Veterinary and Comparative Oncology, 15(1):25-35.
DOI: https://doi.org/10.1111/vco.12139

COMPARISON OF TWO DIFFERENT DOXORUBICIN-BASED CHEMOTHERAPEUTIC PROTOCOLS FOR THE ADJUVANT TREATMENT OF CANINE HEMANGIOSARCOMA

R. Finotello[1,2], D. Stefanello[3], E. Zini[4-6], L. Marconato[7]

[1]Department of Veterinary Clinics, Small Animal Teaching Hospital, University of Pisa, San Piero a Grado, Pisa, Italy

[2]Division of Radiation Oncology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

[3]Department of Veterinary Science and Public Health, University of Milan, Milan, Italy

[4]Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

[5]Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Padova, Italy.

[6]Istituto Veterinario di Novara, Granozzo con Monticello, Novara, Italy.

[7]Centro Oncologico Veterinario, Sasso Marconi, Italy

Dr. Finotello's current address is:

Division of Radiation Oncology

Vetsuisse Faculty, University of Zurich

Winterthurerstrasse 260, 8057 Zurich, Switzerland.

Corresponding author:

Laura Marconato

Centro Oncologico Veterinario

via San Lorenzo 1-4

40037 Sasso Marconi (Bologna), Italy

marconato@centroncologicovet.it

Abstract

Canine hemangiosarcoma is a neoplasm of vascular endothelial origin that has an aggressive biological behavior, with less than 10% of dogs alive at 12 months post diagnosis. Treatment of choice consists of surgery followed by adjuvant doxorubicin-based chemotherapy. We prospectively compared adjuvant doxorubicin and dacarbazine (ADTIC) to a traditional doxorubicin and cyclophosphamide (AC) treatment, aiming at determining safety and assessing whether this regimen prolongs survival and time to metastasis (TTM). Twenty-seven dogs were enrolled; following staging work-up 18 were treated with AC and 9 with ADTIC. Median TTM and survival time were longer for dogs treated with ADTIC compared to those receiving AC (>550 vs 112 days, p=0.021 and >550 vs 142 days, p=0.011, respectively). Both protocols were well tolerated, with no need for dose reduction or increased interval between treatments. A protocol consisting of combined doxorubicin and dacarbazine is safe in dogs with hemangiosarcoma and prolongs TTM and survival time.

Key words: hemangiosarcoma, dog, doxorubicin, dacarbazine, cyclophosphamide

Introduction

Hemangiosarcoma (HSA) is a common tumor in dogs, arising in three different forms: dermal, subcutaneous/muscular and visceral, the latter mainly involving spleen, right atrium or auricle, and liver.[1-3] With the exception of the dermal form, which may behave in a less aggressive fashion, subcutaneous/intramuscular and visceral HSA is a highly malignant cancer, spreading rapidly to lungs, liver, peritoneum and central nervous system.[4,5] Unfortunately, visceral HSA has a silent evolution for a quite long time, and is accompanied by non specific clinical signs. As a consequence, when detected, it is usually in an advanced or metastatic stage, therefore precluding cure.[1,2]

The mainstay of treatment consists of surgery followed by adjuvant intravenous chemotherapy.[6,7] Doxorubicin-based chemotherapy protocols have been administered to dogs with HSA, including doxorubicin as single agent,[6] or combined with ifosfamide,[8] vincristine and cyclophosphamide,[7,9-11] and epirubicin as single agent.[12] Although a three weekly regimen is the commonest schedule administration of doxorubicin, one study attempting to increase dose intensity by more frequent administrations showed such strategy to be well tolerated, although without improved survival time.[13] A metronomic strategy has been also proposed as an alternative treatment, yielding comparable results to conventional dose-intense chemotherapy.[14]

More recently, a combined chemotherapy protocol consisting of doxorubicin, dacarbazine, and vincristine (DAV) was evaluated in metastatic canine HSA.[15] Results suggested that the DAV combination offers clinical response and might prolong survival in dogs with advanced clinical stage HSA. These patients typically have a grave prognosis and treatment options different from hospice or euthanasia are usually discouraged. Thus, the results obtained in the aforementioned study raised interest towards the use of dacarbazine for the treatment of canine HSA. However, significant

haematological and/or gastrointestinal toxicities were observed with DAV, leading to discontinuation of treatment in almost 20% of the patients.[15]

Dacarbazine is a nonclassical alkylating, phase-aspecific agent, which methylates the deoxyribonucleic acid (DNA) at the O_6 methyl group of guanine.[16] Dacarbazine has been previously used in dogs for the treatment of relapsed or resistant lymphomas, high-grade sarcomas, and malignant melanomas, either as single agent or combined with lomustine or doxorubicin.[17-22]

Results of in vitro and in vivo studies suggest that dacarbazine acts synergistically with anthracyclines and has a moderate effect in the treatment of high-grade sarcomas in humans, leading to the need of further investigations of this compound in the treatment of canine HSA.[23-25]

As single agent, a dose up to 1000 mg/m^2 IV over 6-8 hours and 1500 mg/m^2 as a single bolus may be administered to dogs and humans, respectively, every 3 to 4 weeks;[16,19,26-27] however these regimens have been largely replaced in human practice by a daily dose of 250mg/m^2 administered over 5 consecutive days, leading to reduced gastrointestinal toxicity beside similar antitumour activity.[16,28]

In the current study, we prospectively compared adjuvant chemotherapy with doxorubicin and daily dacarbazine boluses to a traditional doxorubicin and cyclophosphamide treatment, aiming at determine safety and assessing whether this regimen prolongs survival time and time to metastasis (TTM) in HSA dogs that underwent surgical intervention.

Materials and methods

Patient eligibility

Client-owned dogs with a surgically removed, histologically confirmed HSA, arising from any abdominal organ or subcutis, were prospectively recruited. Post-surgical investigations included physical examination, haematology, serum biochemistry, abdominal ultrasound and at least two lateral views thoracic radiographs.

Dogs were considered to be at high-risk for developing doxorubicin-related cardiotoxicity if systolic fractional shortening determined by echocardiography was <25%. Dogs with such cardiac function were not enrolled in the study. Dogs with life-limiting diseases different than HSA and those with dermal HSA were also excluded. Dogs were staged according to the World Health Organization (WHO) staging system for domestic animals.[29]

Dogs referred to the institution of one author (DS) were included in Group 1, whereas dogs referred to the institutions of two different authors (LM, RF) were included in Group 2.

Treatment protocol

The objective was to initiate chemotherapy within 7-10 days after surgical intervention. Dogs included in Group 1 were treated with adjuvant doxorubicin (Doxorubicina, Ebewe Italia s.r.l., Roma, Italy) and cyclophosphamide (Endoxan®, Baxter s.r.l., Lurago d'Erba, Como, Italy) (AC), whereas dogs included in Group 2 received doxorubicin and dacarbazine (Deticene®, Aventis Pharma S.p.A, Milano, Italy) (ADTIC).

ADTIC had higher costs compared to AC, and as the study was not supported by a grant, groups could not be randomized. All owners electing ADTIC were asked to sign a written informed consent prior to enrolment.

In either treatment groups, doxorubicin was administered intravenously (IV) at the dose of 30 mg/m^2 every 3 weeks for 4 cycles.

In Group 1, cyclophosphamide was administered orally at 75 mg/m^2 for 4 consecutive days, starting on the day of every doxorubicin administration.

In Group 2, dacarbazine was administered as an IV bolus at the dose of 200 mg/m^2 (without exceeding 250 mg total daily) once daily for 5 days, starting on the day of every doxorubicin administration.

Fractional and summation dose intensities (SDI) for the two protocols are listed in Table 1.[30]

In either group, standard antiemetic therapy consisted in maropitant (Cerenia®, Pfizer, Latina, Italy) administered orally at the dose of 2 mg/kg q24h for 3 consecutive days starting on the first day of chemotherapy. Clavulanate-potentiated amoxicillin (Synulox®, Pfizer, Latina, Italy) was prophylactically administered orally at 12.5-20 mg/kg q12h until the time of the expected neutropenic nadir, and as indicated thereafter. Antibiotic dosage depended on clinician's preference.

A repeated clinical staging work-up consisting of thoracic radiographs and abdominal ultrasound was performed after 2 cycles of chemotherapy. If no local recurrence and/or metastatic disease were observed, the same chemotherapy protocol was continued for two additional cycles. In case of disease progression, a rescue protocol was offered.

Assessment of toxicity

Toxicity resulting from chemotherapy was assessed in Group 1 based on the dog's history, physical examination and complete blood count (CBC) performed 7-10 days after doxorubicin and before the beginning of each cycle, as stated by the Veterinary Co-operative Oncology Group.[31] In Group 2, CBC was checked on day 1, 4, 5, 10 of each chemotherapy cycle. Day 1 was considered as the day of doxorubicin administration.

Statistical analysis

Follow-up and survival times were calculated from the date of diagnosis to the date of last visit or death. For both groups, survival time and TTM (beyond regional lymph nodes), were explored with the Kaplan-Meier product limit method followed by log-rank test. In either group, timing was considered from surgical excision. In the survival analysis, dogs were censored if they were alive at the time of data accrual closure or died of no tumour-related causes, whereas for TTM dogs were censored if, by the last examination, distant metastases had not developed.

To verify whether characteristics of the two treatment groups differed at admission, the Mann Whitney test was used to compare age and body weight, and the Fisher's exact test was used to compare breed (pure- vs cross-breed), sex (male vs female), primary location of the tumor (spleen vs other sites), clinical stage (II vs III) and surgical margins (complete vs incomplete). The latter test was also used to compare the frequency of bone marrow toxicity (present vs absent) that occurred during treatment cycles. $P<0.05$ was considered significant.

Results

Between 2008 and 2014, 27 dogs met the inclusion criteria and were enrolled; 18 (66.6%) of them received adjuvant AC (Group 1), whereas the remaining 9 (33.3%) were treated with ADTIC (Group 2). Features of the dogs are listed in Table 2.

At admission, the two treatment groups did not differ for age, body weight, breed, sex, primary location of the tumour and stage, whereas surgical margins were more often dirty in dogs allocated to receive ADTIC than in those allocated to receive AC [5 of 9 (55.6%) vs. 2 of 18 (11.1%), respectively; p=0.024].

177

178 ***Group 1***

179 There were 9 mixed breed dogs, 2 Boxer, 2 German shepherd, 2 Golden retrievers, 1
180 English setter, 1 Labrador retriever and 1 Italian cane corso. Median age was 9.5 years
181 (range, 6 to 13 years) and median weight was 31 kg (range, 5.2 to 46.4 Kg). There were
182 11 males (n=3 neutered) and 7 female dogs (n=4 spayed). HSA occurred in the spleen
183 as primary site in 15 dogs; 11 of them presented with hemoperitoneum because of
184 splenic rupture. The remaining three dogs had a renal, hepatic and a subcutaneous HSA,
185 respectively.

186 Each dog underwent surgery, consisting of splenectomy, left hepatic lobectomy,
187 nephrectomy or removal of the subcutaneous tumor, according to cancer location.
188 Histopathological evaluation revealed clean surgical margins in the hepatic and
189 subcutaneous HSA; surgical margins were deemed not assessable if the dog was
190 presenting with visceral rupture.

191 According to the TNM classification, 16 were considered having stage II and 2 having
192 stage III HSA. Both dogs with stage III disease had a splenic HSA and macroscopic
193 evidence of metastasis to the omentum; metastatic disease was suspected during
194 celiotomy and this was confirmed through histopathology. Multiple military lesions
195 were observed and metastasectomy could not be performed. No regional
196 lymphadenomegaly and/or other metastatic sites could be documented. Cases are
197 summarized in Table 2.

198 The median time from surgery to the initiation of chemotherapy was 9 days (range, 7-
199 10). The median number of chemotherapy cycles was 4 (range, 2 to 5), with a median
200 cumulative dose of doxorubicin of 120 mg/m^2 and a median cumulative dose of
201 cyclophosphamide of 1200 mg/m^2. The median received SDI for this protocol

202 corresponded to the intended SDI, as none of the dogs required dose reductions and/or
203 treatment delays.
204
205 ***Group 2***
206 There were 6 mixed breed dogs, 1 American Staffordshire terrier, 1 Golden retriever
207 and 1 Labrador retriever. Median age was 9 years (range, 8 to 14 years) and median
208 weight was 26.4 kg (range, 10 to 39.2 kg). There were 3 males (n=1 neutered) and 6
209 female dogs (n=4 spayed). HSA occurred in the spleen as primary site in 5 dogs; all of
210 them presented with hemoperitoneum because of splenic rupture. Two dogs had
211 subcutaneous HSA, one dog had a renal and one had a mesenteric HSA. According to
212 the TNM classification, 6 dogs had stage II (n=2 splenic, n=2 subcutaneous, n=1 renal,
213 n=1 mesenteric) HSA, and 3 dogs had stage III (n=3 splenic) HSA. One dog with
214 subcutaneous stage II HSA had lymphadenomegaly of the ipsilateral regional lymph
215 node; this was surgically excised and metastatic disease was confirmed on
216 histopathology. Two out of the 3 dogs with stage III disease had peritoneal metastases,
217 and 1 had liver metastases. All dogs underwent surgery, consisting of splenectomy,
218 nephrectomy, removal of mesenteric and subcutaneous tumour, according to cancer
219 location; in all cases with stage III disease, metastasectomy was not possible due to the
220 multiple number of metastases. Of the three subcutaneous HSA, 2 were removed with
221 complete margins, whereas one had incomplete margins ($T_2N_0M_0$). Cases are
222 summarized in Table 2.
223 The median time from surgery to the initiation of chemotherapy was 9 days (range, 7-
224 10). The median number of chemotherapy cycles was 4 (range, 2 to 4 cycles), with a
225 median cumulative dose of doxorubicin of 120 mg/m^2 (range, 60 to 120 mg/m^2) and a
226 median cumulative dose of dacarbazine of 4000 mg/m^2 (range, 2000 to 4000 mg/m^2).

The median number of chemotherapy cycles was 4 (range, 2 to 4). The median received SDI for this protocol corresponded to the intended SDI.

Additional treatments

Additional treatments were permitted at the time of development of metastatic disease. These were instituted only in three dogs. In Group 1, a dog with splenic stage II HSA received cyclophosphamide and piroxicam in a metronomic regimen, and in Group 2 a dog with stage II renal HSA and one with splenic stage III HSA received ifosfamide followed by masitinib mesylate.

Clinical outcome

Thirteen (72.2%) out of the 18 dogs in Group 1 developed metastatic disease after a median of 89 days (range, 44 to 188 days). Metastases were found in the liver (n=7), lungs (n=3), peritoneum (n=2), liver and lungs (n=1). The two dogs with metastases to the peritoneum developed haemoabdomen. One (11.1%) out of the 9 dogs included in Group 2 developed pulmonary metastasis after 378 days. The 3 dogs already having metastasis at presentation had disease progression documented after X, Y, and Z days. In these dogs, metastases were found in the lungs (n=1), peritoneum (n=1), kidney and liver (n=1). Overall, median TTM as calculated with Kaplan-Meier product limit was significantly longer for dogs receiving ADTIC compared to those receiving AC (>550 days versus 112 days, respectively; p=0.021; Figure 1).

Sixteen (88.8%) out of the 18 dogs included in Group 1 died by the end of the study: 15 (83.3%) died as a result of HSA progression with a median survival time of 140 days (range, 37 to 301 days), whereas one dog died after 158 days because of gastric dilatation-volvulus with no evidence of tumor recurrence or metastasis. Two dogs with splenic stage II HSA were still alive, 85 and 262 days after the diagnosis.

Seven (77.7%) out of the 9 dogs in Group 2 died by the end of the study: 4 (44.4%) died as a result of HSA progression with a median survival time of 106 days (range, 74 to 480 days). Of these 4 dogs, 3 had splenic stage III HSA and 1 had renal stage II HSA. Regarding the remaining 3, 1 of them (splenic stage II HSA) died 803 days after the diagnosis due to gastric dilatation-volvulus, one (splenic stage II HSA) died after 960 days due to advanced chronic kidney disease (IRIS stage IV), and one (mesenteric stage II HSA) died after 1230 days due to a metastatic mast cell tumour. Two dogs with subcutaneous stage II HSA were still alive, 572 and 1260 days after the diagnosis. Overall, dogs receiving ADTIC had significantly longer median survival than those receiving AC (>550 days versus 142 days, respectively; p=0.011; Figure 2).

In Group 1 none of the dogs was alive at one year after diagnosis whereas in Group 2 the 1 and 1.5 years survival rate was 66.8% and 55.8%, respectively.

Safety

All dogs were evaluated for toxicity. In Group 1, a total of 77 CBCs were evaluated; neutropenia was the only type of bone marrow toxicity, occurring in 6 (33.3%) dogs. Grade 1 neutropenia occurred in 5 dogs, whereas 1 dog developed a grade 3 non-febrile neutropenia. In all dogs neutropenia developed after the first treatment and resolved without sequel. No further hematological toxicities were recorded.

In Group 2, a total of 96 CBCs were evaluated; neutropenia was the only type of bone marrow toxicity, occurring in 7 (77.7%) dogs. Two episodes of grade 1 neutropenia occurred in 1 dog, 6 episodes of grade 2 neutropenia occurred in 5 dogs, 4 episodes of grade 3 neutropenia occurred in 2 dogs, and 1 episode of grade 4 non-febrile neutropenia occurred in one dog. Two dogs developed neutropenia after each cycle, 2 dogs after the third cycle and 2 dogs had only 1 episode of neutropenia during treatment. Among them, 1 developed a grade 2 febrile neutropenia after the second

cycle, which resolved uneventfully after symptomatic treatment. The median number of cycles administered before developing neutropenia was 2 (range, 1 to 2 cycles) and the median number of cycles with dogs showing neutropenia was 3 (range, 1 to 4 cycles). In all dogs neutropenia resolved without sequel.

The frequency of neutropenia was higher in dogs that received ADTIC than in those that received AC (7 of 9 (77.8%) versus 6 of 18 (33.3%), respectively; p=0.046).

Gastrointestinal toxicity was the second most common adverse event in both groups, and consisted of vomiting and decreased appetite. Gastrointestinal toxicity occurred in 7 (38.8%) dogs in Group 1: 2 dogs had grade 1 side effects (one concurrently had grade 1 neutropenia), 4 dogs had grade 2 (2 concurrently had grade 1 neutropenia) and 1 dog had grade 3 toxicity. In every case, a single episode of gastrointestinal toxicity was recorded. In Group 2, 3 (33.3%) dogs developed gastrointestinal toxicity; 2 dogs had grade 1 (1 concurrently had grade 1 neutropenia) and 1 had grade 2 toxicity. The frequency of gastrointestinal side effects did not differ between groups (p=1.000).

Alopecia occurred in one dog in Group 1 at the end of the fourth cycle. No other toxicities were recorded.

Discussion

The treatment of HSA continues to be extremely challenging in veterinary oncology and prognosis for dogs with HSA is poor as a result of aggressive disease, leading to invasion of nearby organs and vessels, early metastasis and limited treatment options providing durable disease control. Surgery is designed to remove all macroscopic tumors and prevent further risk of acute hemorrhage, but is considered purely palliative. The addition of chemotherapy in an effort to treat microscopic disease has been documented to provide a modest improvement in outcome, with reported median

305 survival times in the range of 6-8 months and less than 10% of dogs being alive at 12
306 months.[1,2]
307 In this study, we used a combination of doxorubicin and dacarbazine as adjuvant
308 chemotherapy to determine the safety and efficacy of this treatment in biologically
309 aggressive canine HSA.
310 This study showed that the ADTIC combination is feasible and can be safely
311 administered every 21 days in dogs with HSA, thereby allowing compliance with
312 projected drug doses and scheduled intervals between cycles. All dogs were treated on
313 an outpatient basis, stressing the feasibility of the presently described ADTIC regimen.
314 Side effects were reversible and manageable, with neutropenia being the primary
315 toxicity. One dog experienced febrile grade 2 neutropenia after the second cycle and one
316 asymptomatic grade 4 neutropenia after the third cycle; however they both recovered
317 with supportive care, and subsequent dose reductions were not considered necessary.
318 Notably, none of the dogs developed sepsis due to neutropenia.
319 Overall, the incidence of gastrointestinal toxicity (vomiting and loss of appetite) was
320 low, and no significant differences were observed between Group 1 and 2. We assume
321 that the standard antiemetic medication with maropitant prevented the onset of grade III
322 to IV emesis.
323 Specific guidelines for dose adjustments of antineoplastic agents are not standardized in
324 veterinary oncology; however a 20% to 25% reduction is commonly recommended for
325 the subsequent dose in patients experiencing moderate to severe dose-limiting toxicity
326 (i.e. grade 3-4 toxicity), such as neutropenia or emesis.[32] Excessive toxicity is also more
327 likely to increase treatment-associated costs, have chances of losing owners'
328 compliance and, least but not last, to negatively affect patients' survival. On the other
329 hand, the greatest benefit achievable with anticancer cytotoxic therapy requires a
330 commitment to dose intensity; lack of or reduced dose density have the potential to be

detrimental in cancer treatment, especially in neoplastic diseases known to have the potential of high growth fractions.[33,34] Of course, optimal dose intensity demands therapeutic monitoring in order to either reduce or increase doses based on the patient's capacity to maintain a high quality of life (QoL) during effective therapy. In the mentioned cases, a close monitoring of clinical signs by the clinicians, and detailed owners' information resulted in no lack of compliance. Dogs recovered completely and haematological abnormalities resolved without requiring hospitalization and with no perception of durable decline in QoL by the owner. We therefore elected not to reduce chemotherapy doses at the time of the following cycle, resulting in no effect on the intended SDI of the chemotherapy protocol. Such toxicities did not recur during treatment, being attributable to transient and undiagnosed comorbidities that might have enhanced chemotherapy toxicities, a degree of individual tolerance to chemotherapy, adaption of the owner to gastrointestinal signs or a combination of these. Our approach was based on the thought that dose reductions should not be solely based on the degree of toxicity, but decided on a broader spectrum of variants such as risk of cancer progression, presenting clinical signs and owner compliance. Moreover, cumulative toxicity was also not observed in our study, in fact haematological abnormalities were reversible and the degree of toxicity (either hematological of gastrointestinal) did not increase in the following treatment cycles.

These results differ from a recent publication that reported the use of combined chemotherapy protocol consisting of doxorubicin, dacarbazine, and vincristine (DAV).[15] In that study chemotherapy-related side effects were notable, including several high-grade hematologic and gastrointestinal toxic events. Moreover, almost 20% of the dogs had their protocol discontinued due to chemotherapy-related toxicities; however no treatment-related deaths occurred.[15]

356 This could have multiple explanations. In the current study, the total intended dose of
357 dacarbazine was divided in 5 daily boluses, whereas in the DAV study this was
358 administered as a single dose over 8h infusion; in fact it has been suggested that daily
359 dacarbazine IV boluses may cause reduced gastrointestinal toxicity than slow IV
360 infusions, without negatively affecting antitumour activity.[16,28] Moreover, vincristine
361 was not administered in ADTIC dogs, possibly reducing the risk of gastrointestinal
362 toxicity. It should be also noted that the majority of dogs included in Group 2 presented
363 with no advanced clinical stage, whereas in the DAV study dogs were most likely to
364 have stage III disease; dogs with advanced disease could easily have reduced
365 performance status, potentially leading to enhanced susceptibility to chemotherapy
366 toxicity.[35]
367 No evidence of clinical cardiotoxicity was noted in our study. This finding could be
368 attributed to the entry criteria with regard to cardiac function, the limited number of
369 dogs in the study and/or the low number of doxorubicin treatments administered not
370 reaching the cumulative dose for cardiotoxicity.
371 Despite the small size of this study, our results document an advantage in the use of
372 ADTIC over AC for the treatment of biologically aggressive canine HSA in terms of
373 metastatic control and survival, particularly for stage II HSA. In Group 1, 83% of dogs
374 (AC protocol) where euthanized due to HSA progression with a MST of 142 days,
375 whereas in Group 2 (ADTIC protocol) 44.4% of dogs died due to tumour-related causes
376 with a MST >550days ($p=0.011$); moreover in Group 2 the one and one and a half years
377 survival was achieved in 66.8% and 55.8% respectively, whereas none of the patient
378 reached 1 year survival in Group 2. From our perspective, this data is probably the most
379 relevant supporting further the benefit of ADTIC on patients survival, and may also
380 suggest that a notable proportion of dogs with biologically aggressive HSA may still
381 have a good outcome, if chemotherapy is started in the absence of macroscopic

382 metastatic disease. However, this data should be interpreted carefully as it may be
383 biased by the small number of dogs enrolled in Group 2 and, although debated, to the
384 potentially less aggressive biological behavior of renal and subcutaneous HSA
385 compared to other visceral locations.[36,37] This being said, it must be acknowledged that
386 subcutaneous HSA with the longest diameter >6 cm have been significantly associated
387 with a shorter time to tumour progression and survival time than smaller tumors.[37] In
388 the current study, the 3 subcutaneous HSA measured 8, 6.5 and 12 cm, respectively,
389 supporting the aggressive behaviour and the increased likelihood of developing
390 metastatic disease.
391 Concerning TTM, 66.6% of the dogs treated with AC developed distant metastasis
392 during the study compared to 44% of the dogs treated with ADTIC. Like survival, TTM
393 was significantly longer (p=0.021) if ADTIC was used as adjuvant first-line treatment
394 strategy. This finding may be due to several reasons. Although cyclophosphamide and
395 dacarbazine are both alkylating agents, their antitumor activity differs considerably due
396 to different pharmacokinetic features, lipid solubility, membrane transport properties
397 and specific enzymatic reactions capable of repairing alkylation sites on DNA.[16,38]
398 Cyclophosphamide interferes with DNA replication and transcription of RNA, thereby
399 resulting in disruption of nucleic acid function.[38] Dacarbazine acts by means of
400 alkylation, antimetabolite activity as a purine precursor, and interaction with sulfhydryl
401 groups in proteins.[16] Because the issue of optimizing the treatment strategy to maximize
402 efficacy while limiting toxicity has clinical implications, here we further investigated
403 dose intensity of both adopted protocols by directly comparing cyclophosphamide and
404 dacarbazine. Dacarbazine has greater individual fractional dose intensity when
405 compared with cyclophosphamide, ultimately leading to a greater SDI in combination
406 with doxorubicin. All dogs included in the study received the scheduled doses without

any need for dose reduction, therefore ADTIC treated dogs received a more intense chemotherapy, possibly leading to longer TTM and survival.

Furthermore, beside its cytotoxic activity, dacarbazine has been demonstrated to have in mice antimetastatic property, the underlying mechanism being related to its capacity to enhance tumor immunogenicity.[39,40] In this study, dogs treated with ADTIC had a longer TTM, which may either reflect the capacity of dacarbazine to inhibit metastatic spread or be due to the small sample size of the study.

Limitations of this study include lack of randomization, low number of cases and different tumour site origin. Although subcutaneous and visceral HSA have been described to have an aggressive biological behaviour,[1,3,4,37] little is known about mesenteric HSA.[2] A dog with mesenteric HSA was included in the present study. In addition, 2 dogs included in Group 2 received a rescue protocol after having developed distant metastases, possibly contributing to increased survival.

To conclude, the combination ADTIC was well tolerated and may prolong TTM and survival time in dogs with biologically aggressive HSA, especially if not metastatic at presentation.

References

1. Thamm DH. Hemangiosarcoma. In: *Withrow & MacEwen's Small Animal Clinical Oncology*, 5th ed., SJ Withrow, DM Vail and RL Page eds., St Louis, Saunders Elsevier, 2013: 679-688.
2. Smith AN. Hemangiosarcoma in dogs and cats. *Veterinary Clinics of North America: Small Animal Practice* 2003; **33**: 533-552.

3. Schultheiss PC. A retrospective study of visceral and nonvisceral hemangiosarcoma and hemangiomas in domestic animals. *Journal of Veterinary Diagnostic Investigation* 2004; **16**: 522-526.
4. Shiu KB, Flory AB, Anderson CL, Wypij J, Saba C, Wilson H, Kurzman I and Chun R. Predictors of outcome in dogs with subcutaneous or intramuscular hemangiosarcoma. *Journal of the American Veterinary Medical Association* 2011; **238**: 472-479.
5. Ward H, Fox LE, Calderwood-Mays MB, Hammer AS and Couto CG. Cutaneous hemangiosarcoma in 25 dogs: a retrospective study. *Journal of Veterinary Internal Medicine* 1994; **8**: 345-348.
6. Ogilvie GK, Powers BE, Mallinckrodt CH and Withrow SJ. Surgery and doxorubicin in dogs with hemangiosarcoma. *Journal of Veterinary Internal Medicine* 1996; **10**: 379-384.
7. Wiley JL, Rook KA, Clifford CA, Gregor TP and Sorenmo KU. Efficacy of doxorubicin-based chemotherapy for non-resectable canine subcutaneous haemangiosarcoma. *Veterinary and Comparative Oncology* 2010; **8**: 221-233.
8. Payne SE, Rassnick KM, Northrup NC, Kristal O, Chretin JD, Cotter SM, Kintzer P, Frimberger AE, Morrison-Collister KE, Wood CA and Moore AS. Treatment of vascular and soft-tissue sarcomas in dogs using an alternating protocol of ifosfamide and doxorubicin. *Veterinary and Comparative Oncology* 2003; **1**:171-179.
9. Hammer AS, Couto CG, Filppi J, Getzy D and Shank K. Efficacy and toxicity of VAC chemotherapy (vincristine, doxorubicin, and cyclophosphamide) in dogs with hemangiosarcoma. *Journal of Veterinary Internal Medicine* 1991; **5**: 160-166.

10. Sorenmo KU, Jeglum KA and Helfand SC. Chemotherapy of canine hemangiosarcoma with doxorubicin and cyclophosphamide. *Journal of Veterinary Internal Medicine* 1993; **7**: 370-376.
11. Bulakowski EJ, Philibert JC, Siegel S, Clifford CA, Risbon R, Zivin K and Cronin KL: Evaluation of outcome associated with subcutaneous and intramuscular hemangiosarcoma treated with adjuvant doxorubicin in dogs: 21 cases (2001-2006). *Journal of the American Veterinary Medical Association* 2008; **233**: 122-128.
12. Kim SE, Liptak JM, Gall TT, Monteith GJ and Woods JP. Epirubicin in the adjuvant treatment of splenic hemangiosarcoma in dogs: 59 cases (1997-2004). *Journal of the American Veterinary Medical Association* 2007; **231**: 1550-1557.
13. Sorenmo KU, Baez JL, Clifford CA, Mauldin E, Overley B, Skorupski K, Bachman R, Samluk M and Shofer F. Efficacy and toxicity of a dose-intensified doxorubicin protocol in canine hemangiosarcoma. *Journal of Veterinary Internal Medicine* 2004; **18**: 209-213.
14. Lana S, U'ren L, Plaza S, Elmslie R, Gustafson D, Morley P and Dow S. Continuous low-dose oral chemotherapy for adjuvant therapy of splenic hemangiosarcoma in dogs. *Journal of Veterinary Internal Medicine* 2007; **21**: 764-769.
15. Dervisis NG, Dominguez PA, Newman RG, Cadile CD and Kitchell BE. Treatment with DAV for advanced-stage hemangiosarcoma in dogs. *Journal of the American Animal Hospital Association* 2011; **47**: 170-178
16. Friedman HS, Averbuch SD and Kurzberg J. Alkylating Agents Part B (Methylating agents). In: *Cancer Chemotherapy and Biotherapy: Principles and Practice*, 5th ed., Chabner BA, Longo DL, eds., Philadelphia, Lippincott Williams & Wilkins, 2010: 293-309.

17. Gray KN, Raulston GL, Gleiser CA and Jardine JH. Histologic classification as an indication of therapeutic response in malignant lymphoma of dogs. *Journal of the American Veterinary Medical Association* 1984; **184**: 814–817

18. Van Vechten M, Helfand SC and Jeglum KA. Treatment of relapsed canine lymphoma with doxorubicin and dacarbazine. *Journal of Veterinary Internal Medicine* 1990; **4**:187–191

19. Ahaus EA, Couto CG and Valerius KD. Hematological toxicity of doxorubicin-containing protocols in dogs with spontaneously occurring malignant tumors. *Journal of the American Animal Hospital Association* 2000; **36**: 422–4266

20. Griessmayr PC, Payne SE, Winter JE, Barber LG and Shofer FS. Dacarbazine as single-agent therapy for relapsed lymphoma in dogs. *Journal of Veterinary Internal Medicine* 2009; **23**: 1227-1231.

21. Flory AB, Rassnick KM, Al-Sarraf R, Bailey DB, Balkman CE, Kiselow MA and Autio K. Combination of CCNU and DTIC chemotherapy for treatment of resistant lymphoma in dogs. *Journal of Veterinary Internal Medicine* 2008; **22**: 164-171

22. Dervisis NG, Dominguez PA, Sarbu L, Newman RG, Cadile CD, Swanson CN and Kitchell BE. Efficacy of temozolomide or dacarbazine in combination with an anthracycline for rescue chemotherapy in dogs with lymphoma. *Journal of the American Veterinary Medical Association* 2007; **231**: 563-569

23. Elias AD and Antman KH. Doxorubicin, ifosfamide, and dacarbazine (AID) with mesna uroprotection for advanced untreated sarcoma: a phase I study. *Cancer Treatment Reports* 1986; **70**: 827–833

24. Zucali PA, Bertuzzi A, Parra HJ, Campagnoli E, Quagliuolo V and Santoro A. The “old drug” dacarbazine as a second/third line chemotherapy in advanced soft tissue sarcomas. *Investigational New Drugs* 2008; **26**: 175–181

508 25. Radaelli S, Stacchiotti S, Casali PG and Gronchi A. Emerging therapies for adult
509 soft tissue sarcoma. *Expert review of anticancer therapy* 2014; **14**: 689-704
510 26. Cowan DH and Bergsagel DE. Intermittent treatment of metastatic malignant
511 melanoma with high-dose 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide
512 (NSC-45388). *Cancer Chemotherapy Reports* 1971; **55**: 175-181
513 27. Buesa JM, Gracia M, Valle M, Estrada E, Hidalgo OF and Lacave AJ. Phase I
514 trial of intermittent high-dose dacarbazine. *Cancer Treatment Reports* 1984; **68**:
515 499-504
516 28. Einhorn LH and Furnas B. Combination chemotherapy for disseminated
517 malignant melanoma with DTIC, vincristine, and methyl-CCNU. *Cancer*
518 *Treatment Reports* 1977; **61**: 881-883.
519 29. Owen LN, ed. TNM classification of tumours in domestic animals. Geneva
520 (Switzerland): World Health Organization; 1980
521 30. Longo DL, Duffey PL, DeVita VT Jr, Wesley MN, Hubbard SM and Young RC.
522 The calculation of actual or received dose intensity: a comparison of published
523 methods. *Journal of Clinical Oncology* 1991; **9**: 2042–2051
524 31. Veterinary Co-operative Oncology Group. Veterinary Co-operative oncology
525 group- common terminology criteria for adverse events (VCOG-CTCAE)
526 following chemotherapy or biological antineoplastic therapy in dogs and cats
527 v1.0. *Veterinary and Comparative Oncology* 2004; **2**: 194-213
528 32. Gustafson DL Page RL. Cancer Chemotherapy. In: *Withrow & MacEwen's*
529 *Small Animal Clinical Oncology*, 5th ed., SJ Withrow, DM Vail and RL Page
530 eds., St Louis, Saunders Elsevier, 2013: 157-179
531 33. Lyman GH. Impact of chemotherapy dose intensity on cancer patient outcomes.
532 *Journal of the National Comprehensive Cancer Network* 2009; **7**: 99-108

533 34. Citron ML. Dose-Dense Chemotherapy: Principles, Clinical Results and Future
534 Perspectives. *Breast Care* 2008; **3**: 251-255
535 35. Hayes G, Mathews K, Kruth S, Doig G and Dewey C. Illness severity scores in
536 veterinary medicine: what can we learn?. *Journal of Veterinary Internal*
537 *Medicine* 2010; **24**: 457-66.
538 36. Locke JE and Barber LG. Comparative aspects and clinical outcomes of canine
539 renal hemangiosarcoma. *Journal of Veterinary Internal Medicine* 2006; **20**: 962-
540 967
541 37. Shiu KB, Flory AB, Anderson CL, Wypij J, Saba C, Wilson H, Kurzman I and
542 Chun R. Predictors of outcome in dogs with subcutaneous or intramuscular
543 hemangiosarcoma. *Journal of the American Veterinary Medical Association*
544 2011; **238**: 472-479.
545 38. Gerson SI, Bulgar AD, Weeks LD and Chabner BA. Alkylating Agents Part A
546 (Classical Alkylating Agents). In: *Cancer Chemotherapy and Biotherapy:*
547 *Principles and Practice*, 5th ed., Chabner BA, Longo DL, eds., Philadelphia,
548 Lippincott Williams & Wilkins, 2010: 267-292
549 39. Giraldi T, Houghton PJ, Taylor DM and Nisi C. Antimetastatic action of some
550 triazene derivatives against the Lewis lung carcinoma in mice. *Cancer*
551 *Treatment Reports* 1978; **62**: 721-725.
552 40. Puccetti P, Romani L, Taramelli D, Bonmassar E and Fioretti MC. Drug
553 mediated changes of tumour cell immunogenicity and antigenicity. *International*
554 *Journal of Tissue Reactions* 1982; **4**: 182-189.
555
556
557 **Abbreviated title**: Doxorubicin-dacarbazine for canine hemangiosarcoma
558

559 **Captions to figures:**

560 **Figure 1**: Time to metastases for dogs treated with ADTIC (line) and AC (dots). In the
561 ADTIC group, dogs had a longer time to metastases (>550 days versus 112 days; P=0.021).

562

563 **Figure 2**: Survival time for dogs treated with ADTIC (line) and AC (dots). In the
564 ADTIC group, dogs had a longer survival time (>550 days versus 142 days; P=0.011).

565